

Review

Recommendations for evaluation of bladder and bowel function in pre-clinical spinal cord injury research

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Objective: In order to encourage the inclusion of bladder and bowel outcome measures in preclinical spinal cord injury (SCI) research, this paper identifies and categorizes 1) fundamental, 2) recommended, 3) supplemental and 4) exploratory sets of outcome measures for pre-clinical assessment of bladder and bowel function with broad applicability to animal models of SCI.

Methods: Drawing upon the collective research experience of autonomic physiologists and informed in consultation with clinical experts, a critical assessment of currently available bladder and bowel outcome measures (histological, biochemical, *in vivo* functional, *ex vivo* physiological and electrophysiological tests) was made to identify the strengths, deficiencies and ease of inclusion for future studies of experimental SCI.

Results: Based upon pre-established criteria generated by the Neurogenic Bladder and Bowel Working Group that included history of use in experimental settings, citations in the literature by multiple independent groups, ease of general use, reproducibility and sensitivity to change, three fundamental measures each for bladder and bowel assessments were identified. Briefly defined, these assessments centered upon tissue morphology, voiding efficiency/volume and smooth muscle-mediated pressure studies. Additional assessment measures were categorized as recommended, supplemental or exploratory based upon the balance between technical requirements and potential mechanistic insights to be gained by the study.

Conclusion: Several fundamental assessments share reasonable levels of technical and material investment, including some that could assess bladder and bowel function non-invasively and simultaneously. Such measures used more inclusively across SCI studies would advance progress in this high priority area. When complemented with a few additional investigator-selected study-relevant supplemental measures, they are highly recommended for research programs investigating the efficacy of therapeutic interventions in preclinical animal models of SCI that have a bladder and/or bowel focus.

Keywords: Spinal cord injury, Animal models, Outcome measures, Functional assessment, Bowel, Colon, Bladder, Preclinical studies

Introduction

It is axiomatic that spinal cord injury (SCI) inflicts considerable impact upon all aspects of the life of an individual. Unlike the effects of SCI upon locomotion, profound alterations in autonomic physiology such as

bladder and bowel function have not received a proportional level of scientific urgency.¹ Dysfunction of the somatic and autonomic circuits necessary for successfully evacuating the bladder or bowel, as well as the maintenance of continence until such time as evacuation is desirable, comprises one of the most prevalent and clinically recognized comorbidities of SCI.² These comorbidities, clinically referred to as neurogenic bladder and bowel, affect upwards of 60% of the SCI

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population and a similar percentage of individuals rank neurogenic bladder and bowel (NBB) as a source of major distress.

In March 2017, the Craig H. Neilsen Foundation hosted a workshop with a focus on bladder and bowel dysfunction,¹ as it was and still is a top consumer and research priority.^{3–5} This discussion highlighted the need to identify existing methodologies and measures that could be adopted by laboratories studying animal models of SCI to enhance research in this area and establish the effect of experimental therapeutic strategies on bladder and bowel function.

Anatomical and physiological considerations of the bladder and bowel:

Storage and emptying are common elements affected in SCI individuals with neurogenic bladder and bowel. It is well established that anatomical connections to the smooth muscle of the bladder wall and internal urethral sphincter stem from lumbar sympathetics as well as sacral parasympathetics while a somatic supply from the pudendal nerve supplies the external urethral sphincter. Continence and voiding are dependent on both spinal reflex components that include this efferent supply and primary afferents and spinal interneurons, as well as supra-spinal circuitries (including regions such as cerebral cortex, the periaqueductal gray and pontine micturition center) that modulates it (reviewed by⁶). Interruption of the spinal-bulbo-spinal reflex loop and higher order voluntary control systems following spinal cord injury leads acutely to an areflexic bladder and urinary retention followed chronically by bladder overactivity and inefficient reflex micturition resulting from a loss of coordination between the bladder and its outlet (i.e. detrusor-sphincter dyssynergia). In contrast, colonic physiology and function serves complex integrated processes that differ from the bladder. For example, regional specialization promotes mixing, fluid and electrolyte absorption, and bacterial fermentation within the proximal regions followed by storage and propulsion within more distal regions (see⁷). While colonic anatomy and function is specialized to meet the dietary needs of every organism, the basic neurophysiology of storing and propelling feces remains essentially similar. In health, local enteric motor circuits generate reflexive phasic contractions to promote mixing and slow net distal propulsion of luminal contents. Commonly, SCI provokes prolonged transit times that translate into the excessive reabsorption of water. Giant migrating contractions are large amplitude contractions that occlude the lumen, and propagate without interruption over relatively long distances to produce mass movements; diminished

propulsive contractions are associated with evacuation difficulties.

The need for bladder and bowel measurement recommendations for use in preclinical animal research studies comes in part from a shift in the SCI field to include outcome measures that go beyond common tests of motor systems. For example, the Basso-Beattie-Bresnahan (BBB) locomotor rating scale⁸ has strong validity and is used in many studies that test a therapeutic intervention, but it only assesses motor recovery in rats. An array of functional, histological, biochemical, physiological, electrophysiological and imaging techniques and procedures have been used in preclinical studies specifically designed to assess lower urinary tract (LUT) and bowel dysfunction after acute and/or chronic SCI in multiple species (both small animal and large animal models of SCI). Broadly applicable measures are needed to stimulate research in bladder and bowel function after SCI and while there are many evident trends, no consensus exists on minimum standards.

Recognizing that investigators range from those who use rodents for preclinical efficacy studies to those who seek to directly understand or address the mechanisms of bladder and bowel dysfunction and discover therapeutic targets, the goal of this article is to provide guidance for selection of bladder and bowel measures applicable as either primary or ancillary outcomes. Furthermore, this article provides recommendations concerning the appropriate tools for preclinical assessment of functional recovery. SCI researchers who are new to the areas of bladder and/or bowel function are encouraged to start with two recent introductory reviews.^{6,9}

Methods

A survey of the published SCI literature to identify LUT and bowel outcome measures was done using criteria adapted from a 2006 NIDRR SCI measures working group regarding clinically relevant functional recovery assessment tools in general.¹⁰ Drawing upon the collective research experience of autonomic physiologists, a critical assessment of bladder and bowel outcome measures was made in order to identify the strengths, deficiencies and ease of inclusion for future studies of experimental SCI. History of prior use in preclinical physiological research was required for inclusion. Additional emphasis was given if use in SCI research was evident. The current review primarily targeted methods used over the past 15 years, with inclusion of some standard procedures pre-dating that time. Usage by multiple research groups was given heavy

consideration for inclusion (although for practical reasons, not all groups could be cited), as was experimentation with multiple species of animals. General guidelines for selection of recommendation categories were developed by a sub-group of participants from the March 2017 Craig H. Neilsen Foundation Workshop on bladder and bowel dysfunction after SCI (listed in Appendix 1 of¹). Preclinical outcome measure selection categories for use in SCI studies included ease of use, affordability, time and effort requirements, reliability/reproducibility and bias based on usage, and prior demonstration of sensitivity to change with an intervention (significant differences with $P < 0.05$). Five preclinical recommendation categories were created (Table 1). The recommendations

Table 1. Summarized category recommendations for pre-clinical assessments.

Recommendation Level	Definition	Advantages
Fundamental	An assessment that collects essential and highly relevant information applicable to any study related to NBB.	Demonstrate sensitivity to change Easily incorporated Minimal capital investment
Recommended	An assessment which is essential based on certain conditions or study types in research.	Reasonably incorporated by laboratory or through a core facility Provides a fairly high yield of data for minimal effort Moderate capital investment
Supplemental	An assessment which is essential based on certain conditions or study types in similar area of research.	Reasonably incorporated by laboratory, through collaborations or a core facility. Provides a fairly high yield of mechanistic insight Entails greater training, effort and capital investment.
Exploratory	An assessment that shows promise but is highly novel.	Requires further validation, but may fill current gaps in knowledge. Requires further development and validation Potential to evolve into a recommended tool.
Not Recommended	An assessment that is largely unsuitable for use by most laboratories.	Requires high level of monetary investment or training

are further subdivided based upon whether the primary focus of the preclinical SCI study targets recovery of LUT and/or bowel function versus recommendation as a standard measure(s) where the intervention target is not specific to bladder or bowel management. Additional qualitative criteria were based upon the following properties for each measure:

1. The widespread availability and utilization of a technique by independent investigators that did not directly descend from one common laboratory or mentor.
2. Validity based upon available data utilizing appropriate controls.
3. Sensitivity of a measure to detect changes in acute and chronic pathophysiological models not necessarily including SCI.
4. Preclinical measures that are functionally similar to clinical techniques are identified as such, in contrast to those that are specific to animal models and provide more mechanistic advantages.

Finally, assessment techniques were categorized as 1) histological; 2) biochemical; 3) functional; 4) physiological; and 5) electrophysiological tests.

Results

Each category provides selected examples in order to provide readers with basic familiarity of the assessment strategy.

Lower Urinary Tract (LUT) and Colon (Bowel) Physiology:

The search for studies using methods that assess LUT function in preclinical SCI models indicates an array of techniques and procedures that range from simple collection of urine volume voided to complex recordings of dissociated dorsal root ganglion (DRG) bladder neurons. The results provided in Table 2 reference a small sampling of studies from an array of independent international laboratories that have published SCI studies in recent years containing some aspect of LUT function as an outcome measure.

The number of studies using methods that assess colonic function in preclinical SCI models is considerably smaller. However, the field of neurogastroenterology reflects an abundant number of techniques and procedures that range from measuring fecal output to intracellular recordings of smooth muscle cells and specific populations of autonomic neurons. The results provided in Table 3 reference a number of studies from independent international laboratories that have established techniques outside of SCI studies that are applicable for the understanding of colon function as an outcome measure.

Table 2. Summarized assessment recommendations for pre-clinical studies of lower urinary tract function after SCI.

Measure	Examples of Outcomes	Technique Classification	Evaluation Recommendation for SCI Studies		Reference Examples
			LUT emphasis	Other emphasis	
Tissue Morphology	Hematoxylin and Eosin (H&E) and Masson trichrome of bladder wall; bladder weight; bladder afferents; bladder fibrosis	Histological	Fdtl	Sup	11–23
Ultrasound/Brain Imaging	High resolution images for volumetric calculations	Imaging	Sup	NRec	24–27
Tissue Biomarkers	Immunoassay (ELISA) of bladder tissue (e.g. NGF; cytokines); IHC expression levels of various neuromodulators in bladder afferents (DRG; spinal dorsal horn)	Biochemical	Sup	Sup	6,12,14,15,21,28–33
Immunohistochemistry (IHC) of bladder wall	Neural marker density (PGP9.5; NF200); staining of known receptors/ion channels	Biochemical	Sup	Sup	17,23,30,33–35
RNA extraction and real-time PCR	Total RNA of known targets from the urinary bladder, corresponding DRG and spinal segments	Biochemical	Sup	Sup	20,30,34,36–40
Western blot analysis	Total protein concentrations of known targets in urinary bladder and spinal cord tissues	Biochemical	Sup	Sup	14,20,30,34,41,42
Crede/Reflex Assessments	Presence/absence of voiding; Time to return of reflexive or volitional emptying; volume voided and post-void residual	Functional	Fdtl	Fdtl	12,28,29,34,41,43–50
Urinary Biomarkers	Urine levels of growth factors, cytokines and prostaglandins to assess disease severity and/or treatment response	Functional	Sup/Ex	Sup/Ex	13,14,41
Metabolic Cage	Urine and drink frequency and volume	Functional	Rec	Rec/Sup	15,51–56
Cystometry with or without External Urethral Sphincter EMG	Cystometrogram: resting and peak pressure, bladder contraction amplitude/duration/area under the curve, intercontraction interval, voiding efficiency ($VE = 100 \times (VV / (VV + RV))$); EUS EMG: latency, duration, frequency, amplitude, pattern (tonic/phasic), interburst interval.	Functional	Fdtl	Rec/Sup	11,15–19,29,42,49,54,56–75
Isometric tension recordings	Tone and contractility in bladder smooth muscle strips	Physiological	Sup	NRec	30,76–81
Nerve/ganglion/CNS Recordings	Properties of dissociated labeled bladder afferent neurons; central neuronal responses to stimulation of the bladder	Electrophysiological	Sup	NRec	27,82–85

Fdtl: Fundamental; Rec: Recommended; Sup: Supplemental; Ex: Exploratory; NRec: Not recommended.

Tissue morphological assessments

There are many studies that harvest specific tissues at the completion of the experiments to determine effects of injury processes or interventions. Bladder harvesting is more prevalent than colon yet both provide useful data beginning simply with weight. Weight differences can reflect pathophysiological changes in tissue

composition such as thickening or loss of tissue integrity and is an easily generated outcome measure that should be considered a fundamental element in any study addressing neurogenic bladder or bowel. Both the bladder^{76,138,139} and the colon^{7,140} demonstrate post-SCI alterations in smooth muscle wall thickness and collagen deposition. Collection of the bladder and colon at

Table 3. Summarized assessment recommendations for pre-clinical studies of bowel function after SCI.

Measure	Examples of Outcomes	Technique Classification	Evaluation Recommendation for SCI Studies		Reference Examples
			GI emphasis	Other emphasis	
Tissue Morphology	Hematoxylin and Eosin (H&E) and Masson trichrome of colon wall; crypt integrity; smooth muscle fibrosis. Cecum to anal verge weight.	Histological	Fdtl	Rec	7,86–89
Immunohistochemistry (IHC) of enteric nervous system	Enteric density of fibroblasts and neurons (HuC/D; c-kit/Ano-1; nNOS;ChAT); staining of known receptors/ion channels	Histological	Rec	Sup	7,86,88,90,91
RNA extraction and real-time PCR	Total RNA of known targets from the colon, corresponding dorsal root and nodose ganglia and spinal segments	Biochemical	Sup	Sup	7,92–95
Western blot analysis	Total protein concentrations of known targets from the colon, corresponding dorsal root and nodose ganglia and spinal segments	Biochemical	Sup	Sup	94,96
Metabolic Cage	Fecal output	Functional	Fdtl	Rec/Sup	93,97–99
Fecal analysis	Water; protein; lipid; carbohydrate (macronutrients)	Functional	Rec	Sup	100–103
Fecal analysis	Microbiome	Functional	Sup	Expl	104–108
Transit studies	Whole gut transit of non-absorbable marker	Functional	Rec	Sup	101–103,109
Motility studies	Bead expulsion test from distal colon	Functional	Rec	Sup	110–113
Pressure recordings	Distal or proximal pressure (preferably both)	Functional	Fdtl	Rec	7,114–116
Muscle tension recordings	Tone and contractility in colon smooth muscle (in vivo or ex vivo)	Physiological	Rec	NRec	98,99,103,117–125
Nerve/ganglion/CNS recordings	Pudendal and/or parasympathetic (pelvic) nerve activity or stimulation	Electrophysiological	Sup	NRec	126–129
Neuromuscular recordings	Ex-vivo recording of smooth muscle junction potentials and mucosal/epithelial function	Electrophysiological	Sup	NRec	98,130–132
Enteric ganglion recordings	Ex-vivo intracellular recording of myenteric ganglion neurons	Electrophysiological	Sup	NRec	133–137

Fdtl: Fundamental; Rec: Recommended; Sup: Supplemental; Ex: Exploratory; NRec: Not recommended.

the conclusion of an experiment also permits histological analysis of cryosectioned or paraffin-embedded samples using Hematoxylin and Eosin (H&E) stain to better quantify wall thickness and tissue composition. For more specific mechanistic studies, the assistance of a trained pathologist permits the identification of basic cell types including macrophages, monocytes, lymphocytes & neutrophils (e.g. ^{7,86–89} in Table 3). To distinguish smooth muscle from collagen deposition, the use of Masson's trichrome stain or similar reagent is preferable.^{7,86} Due to the structural and functional differences in the proximal and distal colon, samples from both regions are recommended.^{141,142}

Immunohistochemical assessment of sampled tissues permits the same focused assessments of cell and fiber types and extracellular matrix components. For example, density of the intrinsic motor innervation of the colon by the enteric nervous system can be achieved by immunostaining for the neuron-specific protein

HuC/D or pgp9.5.^{90,96,143} Reductions in enteric neurons have been demonstrated in human archival tissue¹⁴⁰ as well as a rat model of SCI.⁷ While pan-neuronal markers (e.g. HuC/D, pgp9.5) are routinely used to identify myenteric motor neurons and will provide accurate neuronal density assessments, they fail to label the specific neurochemical phenotypes of smooth muscle innervation such as acetylcholine or nitric oxide. Therefore, other specific antibodies are required (see⁹⁶), as cholinergic and nitrergic neurons are among several neuronal phenotypes that are critical for gastrointestinal propulsion.^{88,91,144} Elegant studies mapping the extrinsic innervation have also been performed for the evaluation of efferent and afferent fibers within bladder^{145–147} and gastrointestinal wall.^{148–151}

Molecular/biochemical assessments

For discovery, mechanistic research and target engagement studies, additional tools for analysis of harvested

tissue include the widely-employed evaluation of tissue protein content and mRNA levels of various targeted markers such as inflammation-associated molecules. These may be quantified through RNA extraction, in situ hybridization, or real-time PCR.^{7,11,41,92} Molecular and immunohistochemical techniques are easily combined to cross-validate expression levels with the cellular and sub-cellular localization within target tissues that may be affected by optical limitations to quantify immunopositive pixel densities.

A rapidly advancing and related field is the study of bidirectional interaction between the gut microbiome and the physiology of the entire organism. Intriguing data on the importance of the gut microbiome in the neurotrauma field is emerging,^{104,152} yet caution must be exercised when considering the multifactorial determinants of shifts in the microbiome composition.¹⁵³ For example, microbiome dysbiosis drives systemic endotoxemia and inflammation in a model of SCI¹⁵⁴ as well as proposed reductions in microbiome-derived metabolites.^{155,156} Both of these outcomes have profound effects upon the nervous system.

Functional assessments

Functional assessments provide the most relevant measures of neurogenic bladder and bowel and are considered essential for any studies designed to identify mechanisms of autonomic dysfunction or effects of targeted interventions on these systems. Measures are considered indirect if they reflect the relevant physiological process (i.e. bladder and bowel storage and evacuation) but are influenced by other, indirectly related, processes (drinking and feeding volumes). Conversely, direct measures of these same processes can be achieved by filling or distending the bladder and colon with experimentally-controlled volumes, pressures and rates of stimulation. While these latter approaches can be performed in consenting, unanesthetized humans, the animal models may require an anesthetized subject.

One widely used procedure for small animal SCI studies is the crede procedure (manual emptying of the bladder). Although this is a necessary procedure done throughout the SCI field for post-injury chronic care until the emergence of reflex voiding, its use as an outcome measure is frequently overlooked. The procedure, however, can easily provide quantitative data in the form of the time to reflex void and volume of urine released. Due to the potential for variability in defining reflex voiding and the frequency of manual intervention utilized across studies, careful attention to the published literature is recommended (see⁴³). Daily residual volumes during the acute post-SCI phase of

recovery can be obtained and recorded easily, and have been shown in a number of the studies cited (see [Table 2](#) Crede/Reflex assessments) to be a predictor of long term recovery. The urine expressed is a further potential source of information, as the use of urinary biomarkers continues to expand for the detection and surveillance of a variety of conditions and diseases. Note that in large animals such as the minipig,^{138,157,158} volumes and urine samples can also be readily obtained, as a Foley catheter is kept in place initially post-SCI either until reflex voiding occurs or emptying continued with intermittent catheterization (a common management strategy in human SCI).

Metabolic cage usage for fluid intake and output (equivalent to a voiding diary that is used clinically) has been consistent over the years, although the technology has become more sophisticated, leading to much higher costs. The extensive use of metabolic cages has offered a non-invasive means to simultaneously quantify urine or fecal production^{93,97–99} and composition.¹⁵⁹ One limitation of metabolic cages concerns their use early following SCI when rats undergo the crede procedure (not problematic regarding fecal output). Note that periodic rather than continuous use could limit concern regarding the potential impact of single housing on functional recovery.^{160,161}

Oral administration of a non-absorbable marker (e.g. charcoal, phenol-red; [Table 3](#)) permits a determination of total gastrointestinal transit time, but this time is likely to be influenced by cumulative delays in upper gastrointestinal transit.^{117,162} Colonic passage of a rectally-inserted bead, serving as a traceable fecal pellet, has been employed in multiple research models.^{110–113} This approach avoids upper gastrointestinal transit confounds and permits a distance calculation based upon placement. There is a potential for experimenter-induced confounds stemming from excessive stimulation of the colon wall during insertion or colonic motor activity driven by stress responses. Utilizing the same principles as the clinical application of scintigraphy, radiographic techniques have seen development for use in animal models.^{163,164} These imaging techniques in preclinical SCI models are less common, due to the cost and availability of radiographic equipment.

Physiological assessments

In vivo assessments – The gold standard method used in human urological studies and clinical evaluations, cystometry, appears to be the most common technique used in animal studies with a focus on LUT function. Cystometry also appears in numerous SCI studies that

use a battery of multi-system outcome measures (see Table 2 citations). Cystometric outcome measures that include void contraction amplitude and duration, inter-contraction interval, pressure and voiding efficiency as well as number and amplitude of non-void contractions are done under either awake or anesthetized conditions in species that include mice, rats, rabbits, cats, dogs, sheep and pigs.

Manometric techniques are widely employed in human colorectal evaluation and studies. Variations of this technique are also quite common in the preclinical literature (see Table 3). However, while the temporal resolution and the ability to record propulsive and retro-pulsive contractions along numerous points in the colon is possible with the multi-channel clinical probes, only single channel transducer configurations are commonly employed in preclinical applications. Specifically, preclinical applications often use a rectally-inserted fluid-filled balloon or flexible tube connected to a pressure transducer.^{165–168} Although balloon transducers are easy to use and incur low initial costs, they have several disadvantages regarding both the intrinsic compliance of the balloon wall (latex or scilastic tubing is frequently used) and the large “recording” surface area of the balloon (ca. ≥ 2.5 cm). One alternative solution is the recent re-purposing of miniaturized arterial pressure transducers^{7,116} that can be employed in serial pairings due to a small diameter (e.g. 3 French; ca., ≤ 1 mm).

Ex vivo assessments – Smooth muscle isometric tension of the bladder or colon wall maintained in an *ex vivo* organ bath preparation permits pharmacological investigation of cholinergic^{117,118} as well as non-cholinergic non-adrenergic (NANC) stimulation.^{169,170} Due to the nature of terminal stage experimentation, any *ex vivo* approach would be best suited to assess neuroplasticity or sparing in studies of therapeutic interventions or mechanistic studies therapeutic target development.

Electrophysiological assessments

Often, but not always, cystometry is accompanied by external urethral sphincter EMG activity recording, which provides quantitative information during the filling and voiding phases, including the coordination of detrusor-sphincter activity and quality of bursting patterns during emptying (c.f.,^{29,65,69}). A lack of coordination after SCI for example, referred to as detrusor sphincter dyssynergia, results from tonic sphincter activity which interrupts urine release and prolongs voiding. EMG recording of the external anal sphincter has also been employed.^{171–173} These methods require some training and experience, but once established in

a laboratory, they can be used fairly simply as repetitive or terminal outcome measures.

Additional electrophysiological techniques have been routinely employed in research and are readily adapted to neurogenic bladder and bowel studies. These approaches, however, require a substantially higher level of investment and training. Whole nerve recordings of the autonomic and somatic innervation of the bladder or distal gut, for example, provide quantitative evaluation of segmental afferent and efferent reflex circuits to the viscera (Tables 2 & 3). Intracellular recording in smooth muscle cells for enteric nervous system-mediated junction potentials yields insights to the enteric neurons comprising the final common pathway of smooth muscle contraction.^{98,130,131} Though less common, studies have employed similar recording techniques for the unique electrical activity of bladder smooth muscle cells.^{174,175}

Discussion

The results of this literature methods survey indicate the existence of diverse types of bladder and bowel assessment tools that have been employed in other fields of research and are available for use as outcome measures for studies of SCI, either with or without a primary focus on visceral function.

Lower Urinary Tract (LUT) The most standard and easy/cost-effective outcome measure of LUT function is volume voided and post-void residual measures. Residual volume collections and measurement is recommended at a minimum for all rodent SCI studies during the crede procedure, a necessary standard operating procedure during the acute and sub-acute phases post-injury. Note that the timing for the development of reflexive voiding can vary depending upon severity/completeness of the lesion. Urine measurement/collection beyond the development of reflex voiding would include use of metabolic cages for small animals or intermittent catheterization for large animals such as the minipig.

Another simple procedure is the harvesting of the bladder at the end of the study to obtain its weight. Saving the bladder tissue for morphology assessments is a recommended option for studies with a focus on bladder but can also provide supplemental information for other SCI studies as well. Further supplemental information that can be obtained from bladder tissue includes levels of certain proteins or mRNA.

Cystometry is considered fundamental for most SCI studies with a focus on bladder dysfunction and is recommended for use as a supplement for all SCI studies when bladder function is a question of interest or

target of potential therapy. Although the preference is for awake cystometry and inclusion of external urethral sphincter EMG recording, it is not always practical. Whichever approach is taken (sometimes based upon skill set, timing and convenience), cystometry is recommended as part of a multidisciplinary approach using a combination of different techniques. A few examples of LUT studies investigating a therapeutic intervention using multiple outcome measures including cystometry^{14,15,30,34,40,41} illustrate the benefit of having an array of different methods to demonstrate strong evidence of a therapeutic effect.

Distal Colon Significant differences exist between the functions and the neuromuscular control of the proximal versus the distal colon. The techniques that have been described are applicable to the entire colon but the distal colon offers greater ease of access due to the inherent anatomy of the colon in quadrupedal animal models (rodents, cats, dogs, pigs). For example, in humans the transition from the distal colon to the rectum begins at the distal terminus of the sigmoid colon. The transition to the rectum is less readily visualized in the rat and has been reported as variable lengths (approximately 1–3 cm) relative to the anus.

Tissue harvest along the entire colon represents the highest recommendation as an outcome measure for the beginning stages of a research program on neurogenic bowel. The entire lumen of the gastrointestinal tract undergoes a regular and rapid turnover of the epithelial layer at a rate that surpasses any other tissue. Histological assessment of intestinal morphology provides an index of changes in tissue health within a narrow time frame. The easiest and most cost-effective functional technique that is available for all SCI studies is the transit rate of orally administered non-absorbable dyes. However, pressure measurements of colonic contractions offer greater validity for SCI studies specifically focusing on bowel dysfunction. With proper equipment to eliminate distress, animals can be adapted to unanesthetized recording of distal colon contractions. As with LUT functional studies, an integrated panel of outcome measures will offer stronger insights into deficits and detection of treatment effects.

The trend in many recent SCI studies is inclusion of multiple techniques for targeting one system or multiple measures for many different systems. Although selection of outcome measures is study-specific and dependent upon the level and severity of SCI, there are standard techniques in wide use that are easy to perform at a low cost and not overly time-consuming that should be common to all bladder/bowel-SCI or, ideally, SCI

studies in general. The shortfall in preclinical studies utilizing assessments of bladder and bowel function limits the development of a translational consensus in the field. Adopting these recommendations will help refine those animal models of neurogenic bladder and bowel that reliably predict success in clinical testing.

Acknowledgements

The authors wish to acknowledge support received by the Craig H. Neilsen Foundation for the initial 2017 Bladder and Bowel Workshop that promoted the formation of the functional assessment working group. The authors further acknowledge the American Spinal Injury Association (ASIA) for hosting a luncheon for participants to discuss preliminary findings, during its 2018 annual meeting.

Disclosure statement

No potential conflict of interest was reported by the authors.

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